EXPERIMENTAL VACCINE PROTECTION AGAINST HOMOLOGOUS AND HETEROLOGOUS STRAINS OF FELINE IMMUNODEFICIENCY VIRUS

RUNNING TITLE: Experimental Vaccine Protection Against FIV

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ABSTRACT

The experimental infection of domestic cats with feline immunodeficiency virus (FIV) represents a useful small animal model for testing AIDS vaccine prophylaxis. We previously reported that inactivated whole infected cell or cell-free virus vaccines protected about 75% (11 of 15) immunized cats against intraperitoneal (IP) infection with a low dose of FIV, Petaluma strain. We now report that, using similar but slightly modified FIV vaccine protocols, over 90% of immunized cats were protected against IP infection with the same dose of either homologous FIV (Petaluma strain) (28 of 30 cats) or heterologous FIV (Dixon strain) (27 of 28 cats). These FIV strains differ by 11% in outer envelope amino acid sequences. All control cats (15 of 15) were readily infected with either strain of FIV. Vaccine induced immunity appeared sterilizing and correlated with strong antiviral antibody responses, although not necessarily neutralizing antibody. The adjuvant role of autologous feline T-cellular antigens and duration of immunity in this vaccine model remain to be determined.

Infection of domestic house cats with feline immunodeficiency virus (FIV) is an established cause of fatal immunosuppressive disease under both natural and experimental conditions and is becoming a very useful small animal model for AIDS pathogenesis, antiviral therapy and vaccine testing (13,3). We previously showed that sterilizing immunity against low-dose intraperitoneal (IP) infection with FIV could be achieved in about 75% of cats immunized with inactivated whole FIV vaccines made up of either fixed infected allogenic T-cells or cell-free virus harvested from these cells (20). Protection appeared to correlate in general with vaccine induction of a strong antiviral immune response including neutralizing antibodies. However, the relative titer of neutralizing antibodies did not completely correlate with protection. In contrast to the SIV macaque model (16), immunization of cats with the uninfected T-cells used to grow the virus for vaccine production and challenge, did not protect any cats against challenge infection with FIV (20). Thus, in the FIV model, although the allogeneic T-cell antigens could be shown to contribute an adjuvant effect to the vaccines (20), they did not appear to be the critical correlate of immune protection. In the present study we expanded upon this FIV vaccine model by immunizing a larger number of cats with a slightly modified protocol and by challenging cats protected against the homologous FIV with a heterologous strain of FIV, differing by 11% in outer envelope amino acid sequences.

The IL-2 independent feline T-cell line (FL-4) used for the preparation of the FIV vaccines has been described (18,20). In short, FIV-infected FL-4 cells were fixed in 1.25% paraformaldehyde for 24 hr at 5°C and stored at 5°C ($2.5x10^7$ cells/ml). Equal volumes (0.5 ml) of the cell suspension and adjuvant, adenyl muramyl dipeptide (A-MDP) (500 mg/ml) were mixed immediately prior to vaccination. The cell-free virus preparation consisted of filtered (0.45μ) FL-4 tissue culture fluid pelleted by ultracentrifugation (17000-37000 rpm),

resuspended in phosphate buffered saline (PBS) and treated with 1.25% paraformaldehyde for 24 hr at 5°C. The inactivated virus was resuspended in PBS at a concentration of 500 μg/ml and stored at -70°C. Immediately prior to vaccination, the inactivated virus preparation was thawed at room temperature and mixed with an equal value (0.5 ml) of A-MDP adjuvant. Both fixed-cell and inactivated whole-virus vaccines were shown to be free of infectious virus and viable cells by in vitro virus isolation and proliferation assays and by monitoring vaccinated unchallenged cats for infectious virus using virus isolation and PCR for over 2 years (data not shown).

Forty specific pathogen free (SPF) cats 4-6 months of age were divided into 3 groups: Group A (15 cats) was vaccinated subcutaneously with $2.5x10^7$ fixed infected FL-4 cells per dose plus A-MDP adjuvant, Group B (15 cats) received 250 μg of inactivated whole virus per dose plus A-MDP adjuvant and Group C (10 cats) received adjuvant alone. All cats were given the 1st boost 2 weeks after the primary immunization and the 2nd boost 3 weeks later. Because of apparent A-MDP toxicity, i.e., diarrhea, vomiting, following the primary immunization, the dose of A-MDP was reduced to 125 μg and 10 μg for the 1st and 2nd boosts.

Following vaccination but before challenge with homologous virus (day 0, Figs 1-4) all cats were monitored for their humoral immune response and presence or absence of infectious virus in cultured PBMCs. They remained completely negative for infectious virus by both virus isolation and PCR (Table 1A), further evidence that the vaccines were completely inactivated in vitro. By 3 weeks after the 2nd boost with either vaccine, at the time of homologous virus challenge, substantial levels of antibodies binding to FIV core (p28) and envelope (SU and TM) antigens, as measured by ELISA (1:1000-1:50000 for anti-p28 antibodies) and immunoblot (1:50-1:5000 for anti-SU antibodies) were detected (Figs

1-3). Significant titers of neutralizing antibody (mean titers of 1:50-1:200) were also achieved, generally higher in those cats immunized with the fixed infected cell vaccine (Fig 4). A microculture modification of the virus neutralization (VN) assay using uninfected FeT1 cells (FIV susceptible feline T cells) (20) was used. Unimmunized adjuvant controls had no FIV antibody detected. Three weeks after the 2nd boost, all cats were challenged IP with 10 ID_{50} of the homologous Petaluma strain of FIV, that had previously been titered in vivo (20). For the next 9 months the cats were monitored, as previously described, for infection and latent FIV infection, for FIV antibody response and for clinical status. The results are summarized in Table 1, Part A. All 15 cats immunized with the fixed infected cell vaccine (Group A) remained uninfected and their FIV antibody levels gradually declined over the 9 month interval following challenge. Thirteen of 15 cats immunized with the inactivated whole virus vaccine (Group B) were also completely protected against infection and showed a similar, progressive decline in FIV antibody level. The two cats (249, A40) in Group B that were not protected became persistently infected by 3 weeks after challenge and their FIV antibody levels increased and remained elevated for the next 9 months. All 10 adjuvant control cats seroconverted and became persistently infected within several weeks after challenge.

At 38 weeks after challenge the 15 uninfected cats in vaccine Group A and the 13 protected cats in vaccine Group B were boosted a third time with their respective FIV vaccines. In both groups a marked anamnestic antibody response to FIV core and envelope antigens was elicited (Figs 1-3). Anti-envelope and neutralizing antibody titers peaked higher than the levels reached after the 2nd boost (Fig 3-4). Seven weeks after the 3rd boost the 28 vaccine protected animals were challenged IP with 10 ID_{50} of the heterologous Dixon strain of FIV. This strain differs by 11% and 4%, respectively, in the outer envelope

and transmembrane envelope amino acid sequences, from the FIV Petaluma strain (Fig 5). In vivo titration of this FIV strain in 8 SPF cats (2 per dose from 10^{-1} to 10^{-4}) established that a 10^{-3} dilution of the stock represented 10 ID_{50}. Five of 5 SPF controls were readily infected with this dose of FIV Dixon strain. At 16 weeks after the heterologous FIV challenge all but one of the 28 vaccinated cats in Groups A and B remained uninfected (Table 1B) and showed a steady decline in FIV antibody titers. The single infected cat in vaccine Group A became positive for infectious FIV by 16 weeks post challenge and showed an increase in FIV antibodies. The time of virus isolation suggest that this cat was infected with FIV-Dixon instead of FIV-Petaluma, however further studies (ie. sequencing isolated virus from Cat 346) will be necessary to identify the FIV strain infecting this cat.

These results confirm and extend our previous findings (20) in showing that positive immune protection against homologous FIV challenge infection can be achieved in cats by either inactivated infected cell or cell-free virus vaccines. The increased efficacy noted in the present study (>90% vs 75% in previous study) can probably be attributed to use of a stronger immunogen, i.e., increased number of infected cells and amount of cell-free virus antigen per dose and/or stronger adjuvant preparation. The duration of protection has not yet been tested and the antibodies induced tended to decline rather rapidly, approaching baseline levels by 6-8 months after the last boost. However, the strong anamnestic neutralizing antibody response observed when the animals were re-boosted 10 months after their 2nd boost indicates that both vaccines have induced strong B cell memory. It is not known, however, if such a prompt memory cell response would protect against re-challenge infection in the absence of a recent booster immunization. It does not seem likely that this anamnestic response can be attributed to exposure of these animals to the homologous live

virus challenge given 38 weeks previously because the protection appeared sterilizing, i.e., no evidence of transient or latent infection was observed at that time or thereafter and the antibody levels following this challenge progressively declined. Not only do the current vaccines exhibit enhanced efficacy against the homologous strain of FIV (Petaluma strain) but they also protect very well against IP challenge infection with a heterologous (Dixon strain) FIV, differing by 11% in outer envelope amino acid sequences. This difference is similar to that between the San Diego (PPR), Glasgow and Petaluma strains of FIV (14,15,11). However, this difference is considerably less than the 21% and 22% differences at the external envelope sequences observed between the FIV-Petaluma and the Japanese (FIV-TM2)(10) and Maryland (FIV-MD) (12) isolates, respectively. Experiments are now underway to test for vaccine protection against FIV strains from Japan. Studies are also in progress to determine the variable and conserved regions in the FIV envelope, to map the immunodominant domains in core and envelope proteins and compare sequences among FIV isolates around the world. Preliminary results showing cross-neutralizing antibody activity between different European isolates and FIV-Petaluma (17) suggest that conserved immunodominant regions in the viral envelope may exist in the present vaccines produced from FIV-Petaluma.

The type of sterilization immunity achieved and the parameters of immune protection, including protection against heterologous virus strains, are remarkably similar between the FIV vaccines and the inactivated SIV vaccines. In both FIV and SIV macaque models the mechanism of immune protection achieved with the inactivated whole vaccines remains to be elucidated. In contrast to the HIV-1 chimpanzee model where neutralizing antibodies appear to be protective, a protective effect of neutralizing antibodies in the FIV and SIV models remains uncertain. In the SIV macaque model immunization with the

uninfected human T-cells, used to grow the virus for vaccination and challenge, conferred protection to 2 of 4 monkeys (16), and monkeys protected against SIV grown in the human cells were susceptible to SIV grown in rhesus cells (2). These results suggested that nonviral cellular antigens might be critical determinants of vaccine protection in the SIV macaque system. However, in another SIV macaque model system (SIV_{mne}) protection obtained by vaccination with recombinant SIV gp160 alone correlated with induction of neutralizing antibody in the absence of cellular antibodies (4,7). Yet other studies with recombinant SIV_{mac} env vaccine have failed to yield protective immunity, whether or not neutralizing antibodies were elicited (4). By contrast, in the FIV model the picture appears somewhat clearer in that immunization with the uninfected feline T cells used to grow the vaccine virus does not protect against challenge infection with FIV grown in the same cells (20). Nevertheless, in the FIV model the uninfected allogeneic feline T-cells can be shown to elicit a nonspecific adjuvant effect on the immune response, which does not, however, appear critical to the efficacy achieved (20). In the SIV model, protection by the inactivated whole SIV vaccines correlates more strongly with induction of anticellular antibodies than with induction of antiviral antibodies (9) whereas, in the FIV model, protection correlates at least equally well with antiviral as anticellular antibodies. The few vaccinated cats that failed to be protected against infection have lower antiviral (anti-SU and neutralizing) antibody titers prior to challenge than the protected cats but some vaccinated cats with extremely low levels of neutralizing antibodies were still protected from challenge. Positive passive immunization results using pooled sera from healthy cats infected with FIV (Petaluma strain) (neutralizing titer of 1:400) indicate that antiviral rather than anticellular antibodies are the critical determinant of immune protection (19). The role of vaccine-induced antibody dependent cell cytotoxicity and possibility of enhancing

antibodies in this system are also under investigation.

Initial efforts in Glasgow, UK, to prepare similarly efficacious inactivated whole virus vaccines, using the FIV-Glasgow strain, failed probably because little intact envelope was present in the vaccine and little or no neutralizing antibodies were induced (8). Instead of protection, enhancement of infection was elicited by the vaccine (6). Our efforts to show the presence of enhancing antibodies in the vaccine sera at different dilutions with or without complement on FeT1 cells (feline T cell line used for neutralization assay)(20) were so far unsuccessful (Hohdatsu, T., unpublished observation). Additional studies using a technique used to detect such antibodies in cats infected with feline infectious peritonitis virus (5) are in progress. Investigators from UK are now trying to confirm our positive results using the FIV Petaluma strain grown in FL-4 cells. Development of recombinant FIV envelope vaccines are also underway at both Davis, CA and Glasgow, UK.

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TABLE 1

A

FIV VACCINE PROTECTION AGAINST HOMOLOGOUS AND HETEROLOGOUS FIV CHALLENGES

GROUP	-1 WK PC V[a]	-1 WK PC PCR[b]	3 WK PC V	6 WK PC V	9 WK PC V	16 WK PC V	16 WK PC PCR	37 WK PC V	37 WK PC PCR	# PROTECTED / TOTAL #
GROUP A [N=15] (FIXED INFECTED-CELL VACCINE)	-	-	-	-	-	-	-	-	-	15/15 (100%)
GROUP B [N=15] (INACTIVATED VIRUS VACCINE)	-	-	249[c] A40	249 A40	249 A40	249 A40	249 A40	249 A40	249 A40	13/15 (87%)
HOMOLOGOUS VIRUS CONTROL [N=10]	-	-	55U 55X 354	55U A42 55X JK1 354 7K2 250 DK2 9410	ALL	ALL	ALL	ALL	ALL	0/10 (0%)

B

GROUP	3 WK (48 WK)[d] V	3 WK (48 WK)[d] PCR	5 WK (50 WK) V	5 WK (50 WK) PCR	8 WK (53 WK) V	8 WK (53 WK) PCR	16 WK (61 WK) V	16 WK (61 WK) PCR	# PROTECTED / TOTAL #
GROUP A [N=15]	--	--	--	--	--	--	346	346	14/15 (93%)
GROUP B [N=13]	--	--	--	--	--	--	--	--	13/13 (100%)
HETEROLOGOUS VIRUS CONTROL [N=5]	2MK2 IK2 IK3	2MK2	2MK2 IK2 IK3	2MK2 IK2 IK3	2MK2 IK2 IK3	2MK2 IK2 IK3	2MK2 IK2 IK3 IK4	2MK2 IK2 IK3 2MK6	0/5 (0%)

[a] Virus isolation (V); [b] Polymerase chain reaction (PCR)/Southern blot analysis; [c] Cat identification number; [d] Weeks PC with heterologous virus (Weeks PC with homologous FIV)

LEGENDS

Figure 1. Relative levels of anti-p28 antibodies in sera from vaccinated and unvaccinated-placebo cats were determined by ELISA (20) using electrophoretically-purified p28 as substrate antigen (50 μg/well). Cats were immunized 3x with fixed infected-cell vaccine (▲ △), inactivated whole-virus vaccine (◆ ◇), or placebo prior to homologous FIV-Petaluma challenge at 0 weeks. ELISA was performed at a serum dilution of 1:100 and the results are expressed in O.D. values (20). The dilution titer of vaccinated cats after the 2nd boost ranged from 1:1000 to 1:50000 by immunoblot analysis. Figures 1A and 1B represent results from vaccine protected (◆ ▲) and unprotected (◇ △) cats, respectively, compared to the results from placebo cats infected with homologous (■) and heterologous (□) FIV. Cats A40 and 249 (Fig. 1B, ◇) became virus isolation positive at 3 weeks pc with homologous virus. All vaccine-protected cats (Fig. 1A, ◆ ▲) and 5 naive SPF cats (Fig. 1A,1B, □) were boosted with their respective vaccine or placebo, respectively, at 38 weeks pc and challenged 7 weeks later with heterologous FIV-Dixon. Cat 346 (Fig. 1B, △) became positive by virus isolation and PCR at 16 weeks after heterologous FIV challenge (or 61 weeks pc with homologous FIV). Five cats from each vaccinated and unvaccinated placebo groups were randomly selected to be tested for their anti-p28 antibody titers and the results are expressed as mean with standard deviation. Only those immunized cats which were virus isolation and/or PCR positive were specifically tested for the antibody titers.

Figure 2. Vaccinated and unvaccinated placebo cats were tested for serum antibodies to transmembrane peptide, TM(694-705), by ELISA at a serum dilution of 1:500. The amino acid sequence of this TM peptide is QELGCNQNQFFC. All vaccine protected

cats are shown in Figure 2A along with the two placebo groups infected with either homologous FIV-Petaluma (■) or heterologous FIV-Dixon (□). Cats A40 and 249 (Fig. 2A, ◇) from the inactivated whole-virus group and Cat 346 (Fig. 2B, △) from the fixed infected-cell group were unprotected from FIV challenge.

Figure 3. Vaccinated and unvaccinated placebo cats were tested for serum antibodies to viral surface envelope peptide, SU(595-615), by ELISA at a serum dilution of 1:100. This envelope sequence also published as P100 has been shown to be immunogenic in cats infected with Petaluma isolate (1). The dilution titer of vaccinated cats after 2nd boost ranged from 1:50 to 1:5000 by immunoblot analysis. The description of the animals are the same as above two figures.

Figure 4. Virus-neutralizing antibody titers (VN titers) (20) of vaccinated and unvaccinated placebo cats were tested at -9, 0, 6, 35, 42 54, and 77 weeks after homologous FIV-Petaluma challenge (HoFIV→). Individual VN titers of six to seven cats from the fixed infected-cell (Fig. 4A) and the inactivated whole-virus (Fig. 4B) groups were randomly tested. The two unprotected cats (A40 and 249)(Fig. 4B,□--□,*--*) from homologous FIV challenge and the single unprotected cat (346)(Fig. 4A, x--x) from heterologous FIV challenge were specifically tested for VN titer. Individual VN titers of seven placebo cats challenged with homologous FIV-Petaluma (Fig. 4C,◆◇▲△■ □ *) and two placebo cats challenged with heterologous FIV-Dixon (Fig. 4C,● x) are also shown. Only vaccine-protected cats were given the 3rd vaccine boost on 38 weeks pc (3xB→) and challenged at 7 weeks later with heterologous FIV-Dixon (HeFIV→). Figure D represents the mean VN titers of different groups from Figures A,B, and C and their corresponding viral status. Serum

samples were considered positive for VN titer at tested dilutions when their RT or ELISA-p28 O.D. values were less than those of the positive controls which had no serum antibodies.

Figure 5. The predicted amino acid sequence of *env* gene products from FIV-Petaluma (11), FIV-Dixon, FIV-PPR (14), and FIV-TM2 (10) on lanes 1 through 4, respectively. The six variable regions described by Phillips et al. (14) are indicated by V1 through V6. Potential glycosylation sites are underlined and cysteine residues are boxed. The putative proteolytic cleavage site (▼) separates the surface envelope protein (SU) from the transmembrane (TM) protein. The asterisk (*→) represents the location where additional glycine (G) resides for FIV-TM2.

Fig. 1

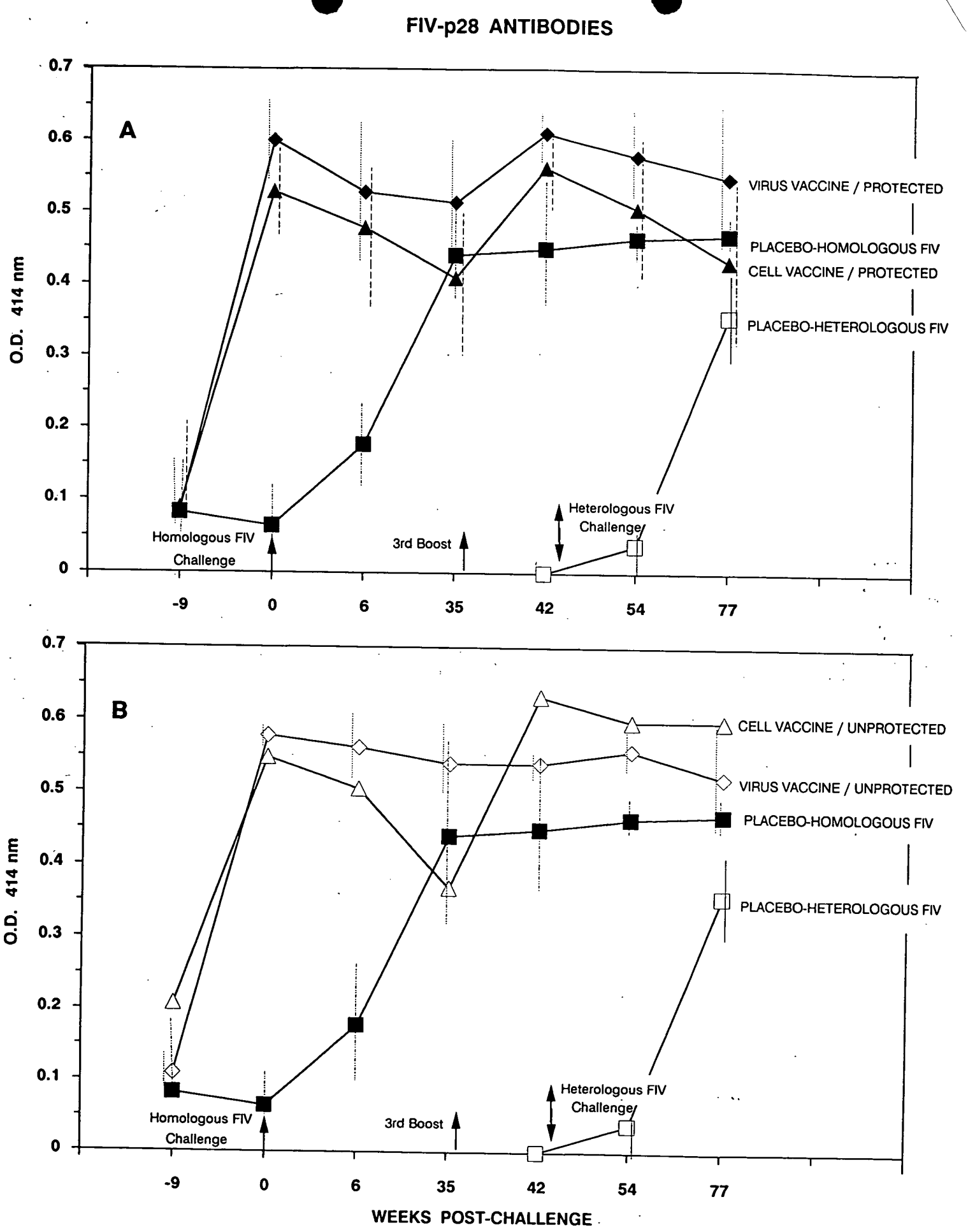
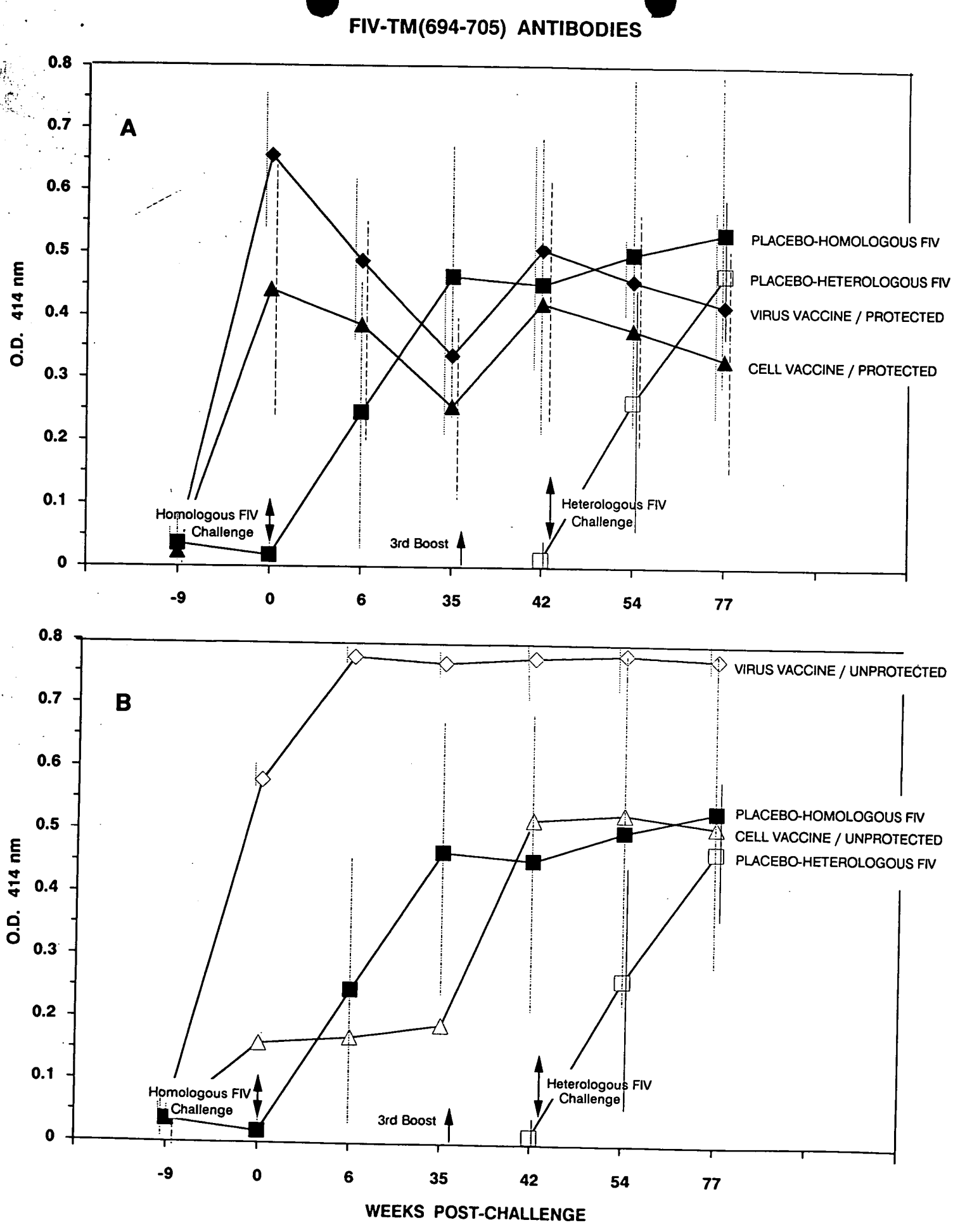

FIV-p28 ANTIBODIES
A
B
O.D. 414 nm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
VIRUS VACCINE / PROTECTED
PLACEBO-HOMOLOGOUS FIV
CELL VACCINE / PROTECTED
PLACEBO-HETEROLOGOUS FIV
CELL VACCINE / UNPROTECTED
VIRUS VACCINE / UNPROTECTED
Homologous FIV Challenge
3rd Boost
Heterologous FIV Challenge
-9
0
6
35
42
54
77
WEEKS POST-CHALLENGE

Fig. 2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B
O.D. 414 nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-9
0
6
35
42
54
77
PLACEBO-HOMOLOGOUS FIV
PLACEBO-HETEROLOGOUS FIV
VIRUS VACCINE / PROTECTED
CELL VACCINE / PROTECTED
VIRUS VACCINE / UNPROTECTED
CELL VACCINE / UNPROTECTED
Homologous FIV Challenge
3rd Boost
Heterologous FIV Challenge
WEEKS POST-CHALLENGE

Fig. 3

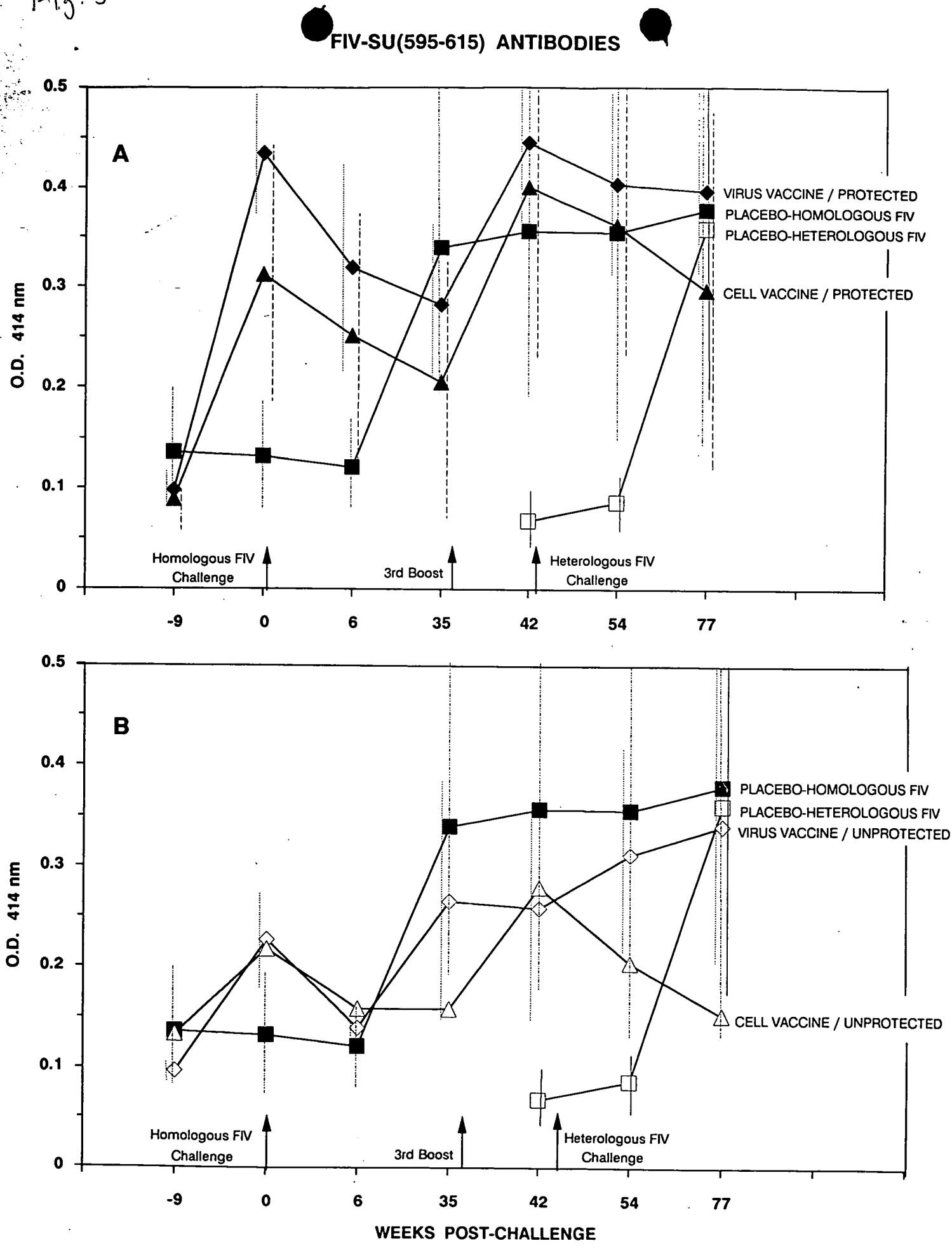

FIV-SU(595-615) ANTIBODIES
A
B
O.D. 414 nm
0.5
0.4
0.3
0.2
0.1
0
VIRUS VACCINE / PROTECTED
PLACEBO-HOMOLOGOUS FIV
PLACEBO-HETEROLOGOUS FIV
CELL VACCINE / PROTECTED
PLACEBO-HOMOLOGOUS FIV
PLACEBO-HETEROLOGOUS FIV
VIRUS VACCINE / UNPROTECTED
CELL VACCINE / UNPROTECTED
Homologous FIV Challenge
3rd Boost
Heterologous FIV Challenge
-9
0
6
35
42
54
77
WEEKS POST-CHALLENGE

Fig. 4

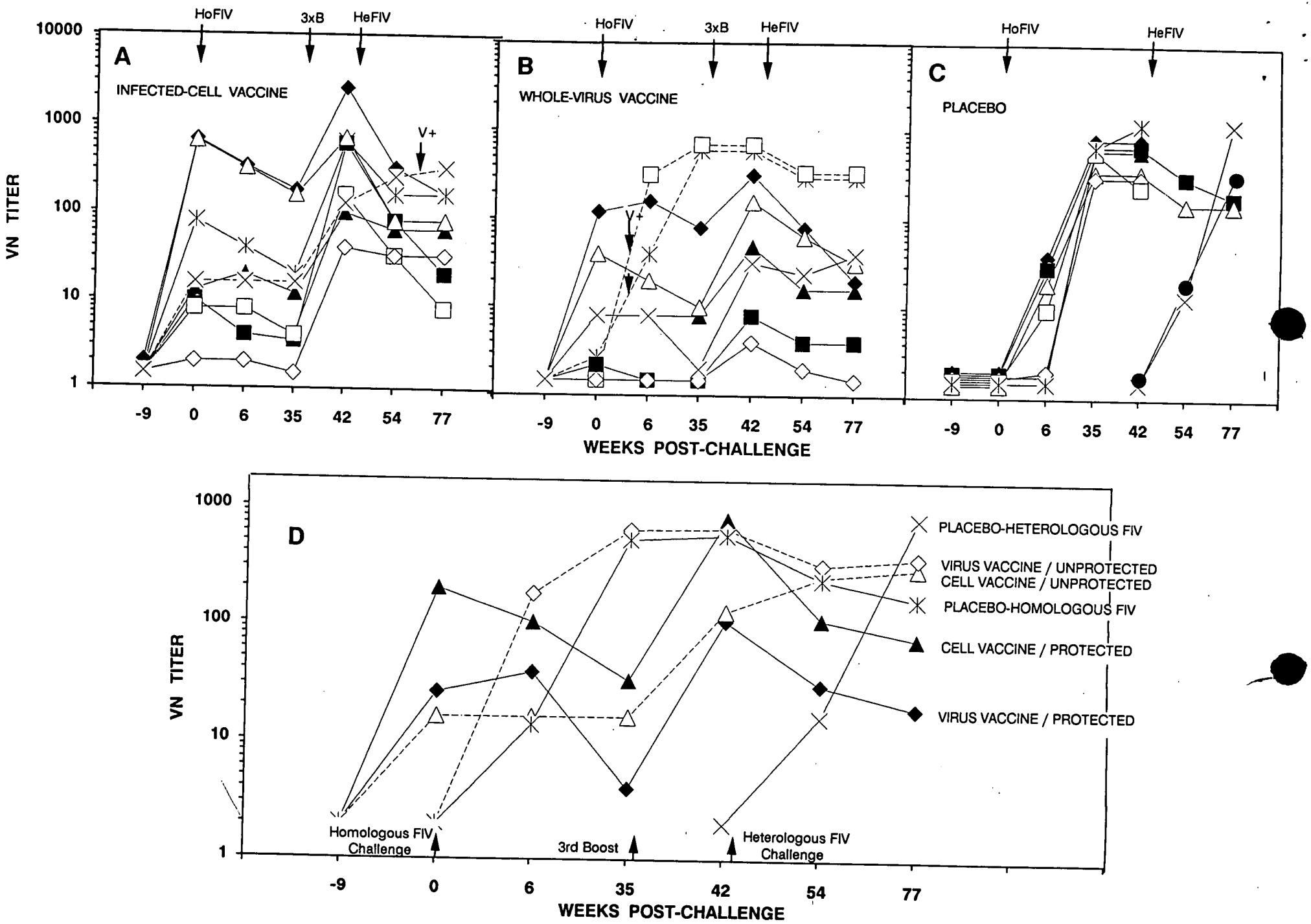
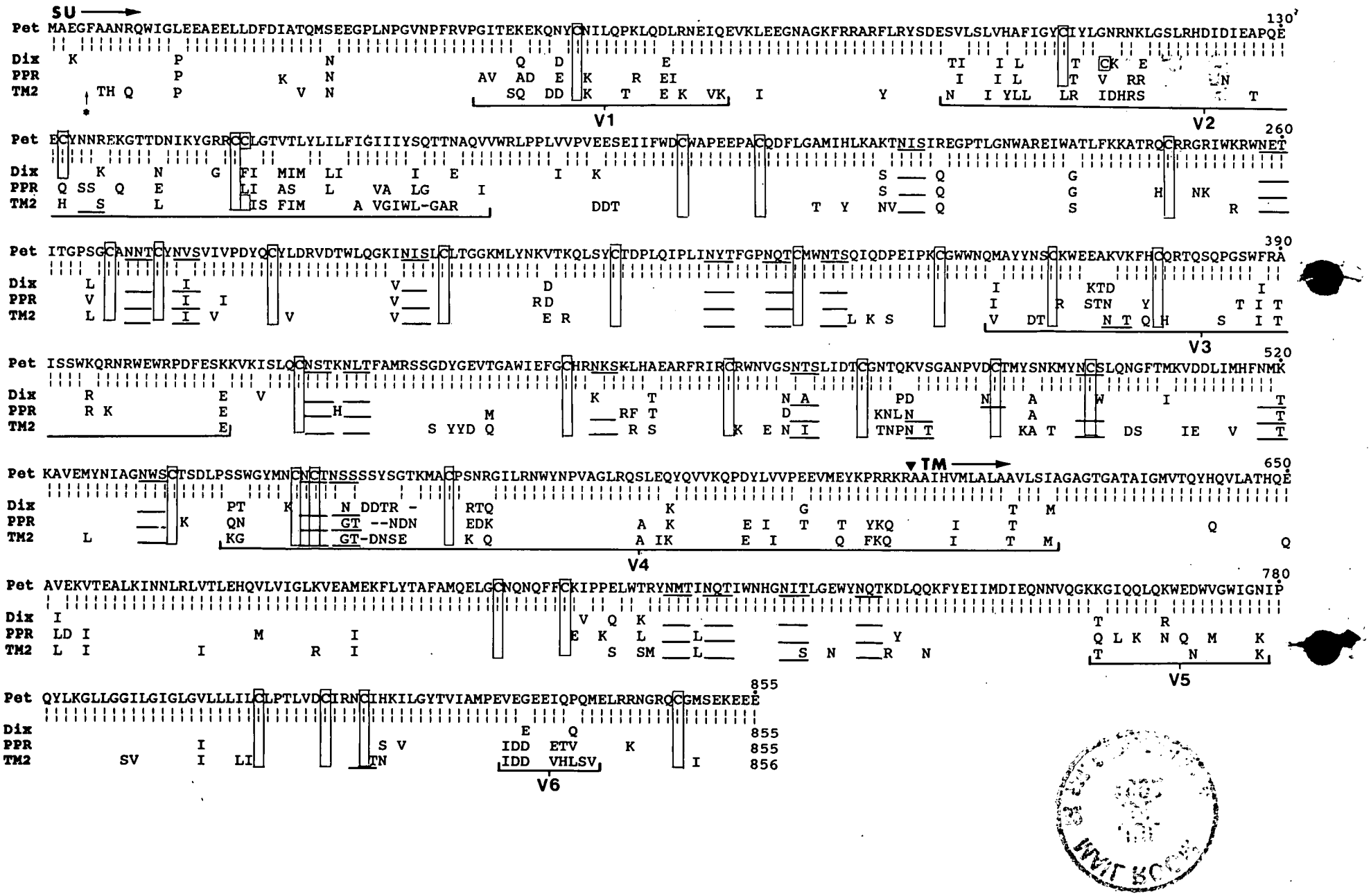

HoFIV
3xB
HeFIV
A
INFECTED-CELL VACCINE
B
WHOLE-VIRUS VACCINE
C
PLACEBO
V+
VN TITER
10000
1000
100
10
1
-9
0
6
35
42
54
77
WEEKS POST-CHALLENGE
D
PLACEBO-HETEROLOGOUS FIV
VIRUS VACCINE / UNPROTECTED
CELL VACCINE / UNPROTECTED
PLACEBO-HOMOLOGOUS FIV
CELL VACCINE / PROTECTED
VIRUS VACCINE / PROTECTED
Homologous FIV Challenge
3rd Boost
Heterologous FIV Challenge

Fig. 5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Dix
PPR
TM2
V1
V2
Pet ECYNNREKGTTDNIKYGRRCCLGTVTLYLILFIGIIIYSQTTNAQVVWRLPPLVVPVEESEIIFWDCWAPEEPACQDFLGAMIHLKAKTNISIREGPTLGNWAREIWATLFKKATRQCRRGRIWKRWNET
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V3
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TM
V4
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V5
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V6